Comparative in vivo Adulticidal Activity of a Topical Dinotefuran versus an Imidacloprid-Based Formulation against Cat Fleas (*Ctenocephalides felis*) on Cats*

Martin Murphy, MVB, MAnSc, PhD, MRCVS, CBiol, MIBiol, MRQAA
Cathy Ann Ball, VMD, MSb,†
Sheila Gross, PhDb

a Charles River Laboratories Preclinical Services Ireland, Ltd.
Carrentrila, Ballina, Co. Mayo
Ireland

b Summit VetPharm
400 Kelby Street
Fort Lee, NJ 07024

**CLINICAL RELEVANCE**

Fleas cause significant discomfort to pet cats and distress to their owners and are also vectors of disease; severe infestations can cause anemia or flea allergy dermatitis and can lead to infections with *Dipylidium caninum* and *Bartonella henselae*. Rapid flea kill is an important feature of flea preventives. The efficacy of dinotefuran (Vectra for Cats & Kittens, Summit VetPharm) was compared with that of imidacloprid (Advantage, Bayer Animal Health) against *Ctenocephalides felis* when applied topically once on day 0. Cats were infested with 100 (±3) *C. felis* on study days −1, 8, 15, 22, and 29. Live fleas were counted on study days 0 (2, 6, and 12 hours after treatment), 9, 16, 23, 29 (2, 6, and 12 hours after infestation), and 30. Cats treated with dinotefuran had significantly (*P* < .05) fewer fleas than the control cats at all posttreatment examinations except day 29 at 2 hours after infestation and significantly (*P* < .05) fewer fleas than cats treated with imidacloprid on days 0 (2 hours after treatment), 9, 16, 23, 29 (6 and 12 hours after infestation), and 30.

**INTRODUCTION**

The neonicotinoids are an important class of compounds used to target adult fleas. Neonicotinoids are safe and effective insecticides that disrupt the insect nervous system by mimicking the action of acetylcholine on postsynaptic

*This research was sponsored by Summit VetPharm, Fort Lee, NJ. The results were presented at the 53rd Annual Meeting of the American Association of Veterinary Parasitologists, July 19–22, 2008, New Orleans, LA.
†Correspondence should be sent to Dr. Ball: fax, 201-585-9525; email, cball@summitvetpharm.com.
nicotinic acetylcholine receptors. In the insect central nervous system, acetylcholine receptors are ionotropic receptors that form ligand-gated ion channels in the nerve cell’s plasma membrane when bound with the neurotransmitter acetylcholine. The binding of acetylcholine to the insect acetylcholine receptors causes the channels to open and Na⁺ to move in. When enough Na⁺ has entered the cell, the nerve cell depolarizes. Acetylcholinesterase bound to the postsynaptic membrane breaks down the acetylcholine, stopping the nerve impulse. When bound to the acetylcholine receptors, neonicotinoids cannot be broken down by acetylcholinesterase, thereby keeping Na⁺ channels open and prolonging the nerve impulse. Fleas affected by neonicotinoids show uncontrollable tremors and ineffective movements and eventually die.¹

The compounds in the first-generation neonicotinoid class, imidacloprid and nitenpyram, were based on the molecule nicotine. These compounds have side chains with a chlorinated aromatic pyridine ring. Dinotefuran, a third-generation, rapid-acting nitroguanidine neonicotinoid insecticide, also exerts action on the acetylcholine receptor, but it is unique in that its structure is based on the acetylcholine molecule, not nicotine. Structurally, dinotefuran is a nonchlorinated, nonaromatic compound that does not bind to the same sites on the acetylcholine receptor as imidacloprid and other neonicotinoids. Instead, dinotefuran is thought to bind at a unique site on the receptor in the insect nerve synapse.²

Neonicotinoids are selective for insect acetylcholine receptors and have little to no affinity for mammalian acetylcholine receptors. A unique cationic site on insect acetylcholine receptor subunits is associated with the electronegative core of neonicotinoid molecules. Mammalian acetylcholine receptors do not contain this site. Acetylcholine receptors are classified as nicotinic or muscarinic. Nicotinic receptors predominate in the insect nervous system, which is why neonicotinoids are so effective against insects, whereas muscarinic acetylcholine receptors predominate in the mammalian nervous system.¹³ The difference in the structures of insect versus mammalian acetylcholine receptors—the selectivity of receptors and the mechanism of detoxification—makes neonicotinoids in general and dinotefuran in particular safe to use for flea control in cats. The relatively high water solubility and slow metabolism of neonicotinoids, especially dinotefuran, in mammals allows the molecules to be excreted unchanged in urine.² Dinotefuran has an excellent toxicology profile in mammals, fish, and birds. The acute oral median lethal dose for dinotefuran is greater than 2,000 mg/kg in rats, the highest of all neonicotinoids used in flea-treatment products. The compound has been studied in mice, rats, rabbits, guinea pigs, dogs, and wild birds (mallards, Japanese quail, northern bobwhite quail). The Environmental Protection Agency concluded that dinotefuran is safe in the home environment.⁶

The insect growth regulators (S)-methoprene and pyriproxyfen target the insect endocrine system, specifically juvenile hormone (JH) activity. JH regulates the molting of insects from one developmental stage to the next,
and high concentrations of JH prevent molting to the next stage. In insects, the enzyme JH esterase normally degrades JH, and molting occurs as JH levels drop. Pyriproxyfen mimics the actions of JH but is not broken down by JH esterase and arrests the development of flea eggs (ovicidal), flea larvae (larvicidal), and early (pharate) pupae. During these developmental phases, when there is sensitivity to JH, pyriproxyfen prevents the activation of genetic switches in the development sequence of the cells necessary for progression to the next stage. Adult cat fleas exposed to pyriproxyfen 24 hours before laying their eggs deposited eggs that were devoid of yolk, were weak, and collapsed shortly after being laid. By 70 hours after initial exposure, eggs laid had some cells, but there was no organization or a cleavage center; therefore, no blastoderm was formed. Because of its stability in ultraviolet light, pyriproxyfen helps prevent both indoor and outdoor environmental infestations. Pyriproxyfen also has an excellent toxicologic profile in mammals (mice, rats, rabbits, dogs, lactating goats).

**TABLE 1. Treatment Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Active Ingredient</th>
<th>Volume (ml)</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 8)</td>
<td>Control</td>
<td>None</td>
<td>0.8</td>
<td>Topical</td>
</tr>
<tr>
<td>2 (n = 8)</td>
<td>Vectra for Cats &amp; Kittens</td>
<td>22% dinotefuran + 3% pyriproxyfen</td>
<td>0.8</td>
<td>Topical</td>
</tr>
<tr>
<td>3 (n = 8)</td>
<td>Advantage (Bayer Animal Health)</td>
<td>9.1% imidacloprid</td>
<td>0.4</td>
<td>Topical</td>
</tr>
</tbody>
</table>

Cats & Kittens formulation for a full 30 days after treatment. To assess the clinical proficiency of Vectra for Cats & Kittens, this study was designed to measure the speed of kill against cat fleas after treatment with Vectra for Cats & Kittens compared with a product containing a first-generation neonicotinoid, imidacloprid (Advantage, Bayer Animal Health).

**MATERIALS AND METHODS**

Thirty healthy cats (15 males and 15 females) were preconditioned, and 24 cats (12 males and 12 females) were allocated to the study. The age and weight ranges were 8 to 19 months and 2.4 to 3.9 kg, respectively, for the males and 9 to 17 months and 2.5 to 3.2 kg, respectively, for the females. To determine the cats’ ability to host flea infestations, each cat was infested with 100 fleas on day –3. On day –2, the cats were combed and fleas counted. A cat had to retain at least 50% of the fleas applied to qualify for inclusion in the study. The assignment of animals to treatment groups was carried out on day –2 using the following procedure: within sex, animals were ranked initially by body weight from heaviest to lightest and then by day –2 flea counts from highest to lowest. The first three animals within each sex formed a replicate. Within each replicate, animals were assigned to one of three study groups using random order numbers derived from Fisher and Yates tables. This procedure continued until 24 animals were allocated to the study groups (eight cats/group; Table 1).
On day –1, all cats were infested with 100 (±3) one- to two-week old European-sourced unfed adult *Ctenocephalides felis* of mixed sex ratio (35% to 65% females and 35% to 65% males) from Charles River Laboratories Preclinical Services Ireland, Ltd. (Carrentrlia, Ballina, Co. Mayo, Ireland). Fleas were supplied in vials containing 100 (±3) fleas/vial. Flea infestations were accomplished by placing fleas on the cat’s lumbosacral region and allowing them to disperse within the hair. The same procedure was followed for subsequent infestations on days 8, 15, 22, and 29.

On day 0, the cats in groups 2 and 3 were treated with the commercially packaged products according to the manufacturers’ instructions. Vectra for Cats & Kittens (lot RDZ-008) was supplied by Summit VetPharm in the commercial sleeve package containing the sealed clamshell that holds the patented applicators. Advantage (lot KP04N85) was purchased from Bayer Animal Health and was supplied in a box containing foil-sealed application tubes. Group 1 cats served as controls and received a placebo solution containing the Vectra for Cats & Kittens vehicle only, which was applied using a needleless syringe. All control and treatment items were inventoried (with lots and amounts recorded), packed at Summit VetPharm, and shipped (with a climate control request displayed on the package) directly to the study director (M.M.) at Charles River Laboratories Preclinical Services Ireland, Ltd. On arrival, the shipment received was checked against the inventory packed and was stored at room temperature in the climate-controlled, limited-access test article storage room until used in the treatments.

Both products and the placebo control were administered by parting the hair at the application site on the back of the head where it meets the neck and applying the entire dose directly to the skin of the cat.

Fleas were collected and counted from each animal at the following time points:

**Day 0:** 2 hours (±10 minutes), 6 hours (±15 minutes), and 12 hours (±20 minutes) after treatment

**Day 9:** 24 (±2) hours after infestation on day 8

**Day 16:** 24 (±2) hours after infestation on day 15

**Day 23:** 24 (±2) hours after infestation on day 22

**Day 29:** 2 hours (±10 minutes), 6 hours (±15 minutes), and 12 hours (±20 minutes) after infestation on day 29

**Day 30:** 24 (±2) hours after infestation on day 29

The number of live fleas on each animal was determined by combing the cats. Each cat was combed for a minimum of 15 minutes using a flea comb. The entire coat was combed thoroughly, with special attention given to the neck region, around the ears, and between the front and back legs, all areas known as preferential flea feeding sites. All hair removed during combing was thoroughly searched for the presence of fleas. To determine if the fleas were alive and viable, the fleas along with the hair from the cats were placed on the table and observed for purposeful movements. If viable fleas were found in the last 5 minutes of combing, the cat was combed for an additional 5 minutes. The number of live viable fleas was counted and recorded. Live fleas were returned to the cat after the 2- and 6-hour counts on day 0 and after the 2-, 6-, and 12-hour counts on day 29. Fleas were discarded after the 12-hour count on day 0 and after the counts on days 9, 16, 23, and 30.

All personnel involved in any data collection, flea counts, or clinical assessments were blinded to treatment group. The control and treatment groups were color coded, and each cat had its own flea comb, which was identified by color.
and cat identification number. All personnel handling the cats for any study activity, combing, assessment, feeding, or cleaning wore disposable laboratory gowns, gloves, and hats, which were changed between color groups.

Percentage reduction in flea counts was defined by the group mean live flea reduction compared with the control group at all time points after treatment. Live flea counts were transformed to the natural logarithm of (count + 1) to compute geometric means. Percent reduction in flea count for each treated group and time point was calculated according to the following equation, in which \( GMC \) is the geometric mean number of fleas in the control group and \( GMT \) is the geometric mean number of fleas in the treatment group:

\[
\text{% Reduction in Flea Counts} = 100 \times \frac{(\text{GMC} - \text{GMT})}{\text{GMC}}
\]

The control group was compared with each treated group using a \( t \)-test for means with poolable variances or for means with unequal variances, as appropriate. Variances were compared using an \( F \) test, and Satterthwaite’s approximation was used to determine the degrees of freedom for the unequal-variance tests; where all values were zero for one treatment group, variances were declared unequal, by definition. The Vectra for Cats & Kittens group was compared with the Advantage group according to the same procedures.

Throughout the study, cats were individually housed in cages measuring approximately 0.2 m\(^2\) and covered with rubber matting served as a resting area for the cat. Rubber matting also covered the floor of each cage. A toy was made available to each animal in its cage. A litterbox containing wood shavings was provided in each cage throughout the study. When cats were infested with fleas, a nest box containing no shavings was provided to each cat. Routine feeding, watering, and cleaning were performed according to the laboratory’s current standard operating procedures. The husbandry conditions under which the cats were maintained were in compliance with Statutory Instrument S.I. No. 556 of 2002, the legal document that incorporates EC directive 86/609/EC into Irish law. Temperature and humidity level of the housing unit were measured daily; the temperature remained between 17°C and 22°C and the relative humidity between 36% and 61% for the duration of the study. Each cat was interacted with socially for a minimum of 2 minutes daily, excluding weekends and public holidays. Social interaction included either rubbing, stroking, talking to, or playing with the animal in its cage. No animal interaction was performed on day 0 or on days when scheduled weighing, flea infestation, and flea combings took place. Standard commercially available cat food was fed to the adult cats at the recommended rates (i.e., approximately 100 g/cat/day). Cats were fed once daily; the quantity of diet offered and consumed was not recorded. The study was conducted with approval of Charles River Laboratories Preclinical Services Ireland, Ltd., IACUC.

** Vectra for Cats & Kittens demonstrated the ability to kill 98% or more of cat fleas by 6 hours after treatment and for up to 30 days. **
Table 2. Summary of Geometric Mean Flea Counts and Percent Reduction in Flea Count

<table>
<thead>
<tr>
<th>Day</th>
<th>Control (0.8 ml)</th>
<th>Vectra for Cats &amp; Kittens (0.8 ml)</th>
<th>Advantage (0.4 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–2</td>
<td>69.1</td>
<td>73.3</td>
<td>71.9</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td>85.4</td>
<td>13.7&lt;sup&gt;a,b&lt;/sup&gt; (83.9)</td>
<td>83.3 (2.5)</td>
</tr>
<tr>
<td>6 hr</td>
<td>85.6</td>
<td>0.0&lt;sup&gt;ab&lt;/sup&gt; (100)</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt; (98.8)</td>
</tr>
<tr>
<td>12 hr</td>
<td>83.9</td>
<td>0.0&lt;sup&gt;c&lt;/sup&gt; (100)</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt; (99.9)</td>
</tr>
<tr>
<td>9</td>
<td>75.2</td>
<td>0.0&lt;sup&gt;a,b&lt;/sup&gt; (100)</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt; (96.5)</td>
</tr>
<tr>
<td>16</td>
<td>84.0</td>
<td>1.2&lt;sup&gt;a,b&lt;/sup&gt; (98.5)</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt; (90.0)</td>
</tr>
<tr>
<td>23</td>
<td>87.7</td>
<td>1.4&lt;sup&gt;a,b&lt;/sup&gt; (98.4)</td>
<td>9.8&lt;sup&gt;a&lt;/sup&gt; (88.8)</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td>94.9</td>
<td>55.6 (41.4)</td>
<td>77.8&lt;sup&gt;a&lt;/sup&gt; (18.0)</td>
</tr>
<tr>
<td>6 hr</td>
<td>85.1</td>
<td>4.3&lt;sup&gt;a,b&lt;/sup&gt; (95.5)</td>
<td>36.8&lt;sup&gt;a&lt;/sup&gt; (56.7)</td>
</tr>
<tr>
<td>12 hr</td>
<td>74.4</td>
<td>0.9&lt;sup&gt;a,c&lt;/sup&gt; (98.8)</td>
<td>7.4&lt;sup&gt;a&lt;/sup&gt; (90.1)</td>
</tr>
<tr>
<td>30</td>
<td>68.4</td>
<td>0.3&lt;sup&gt;a,c&lt;/sup&gt; (99.6)</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt; (96.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly different from control (<i>P</i> < .01).
<sup>b</sup>Significantly different from Advantage (<i>P</i> < .01).
<sup>c</sup>Significantly different from Advantage (<i>P</i> < .05).

**RESULTS**

No adverse effects were observed in any cats during the study. For the control group, geometric mean flea counts ranged from 83.9 to 85.6 at 2, 6, and 12 hours after placebo treatment; this group also retained geometric mean flea counts of 74.4 to 94.9 on day 29, when fleas were counted and returned to the cat at 2 and 6 hours (Table 2).

Cats treated with Vectra for Cats & Kittens had significantly (<i>P</i> < .01) fewer fleas than control cats at all posttreatment examinations except for one time point, 2 hours after infestation on day 0. Percent reduction in flea count was never 100% with Advantage at any posttreatment examination but was at least 90% on days 0 (at 6 and 12 hours after treatment), 9, 16, 29 (at 12 hours after infestation), and 30 (Figure 1).

Cats treated with Vectra for Cats & Kittens had significantly fewer fleas than cats treated with Advantage on days 0 (at 2 hours after treatment), 9, 16, 23, and 29 (at 6 hours after infestation) (<i>P</i> < .01) and on days 29 (at 12 hours after infestation) and 30 (<i>P</i> < .05). On day 0 at 2 hours after treatment, flea counts ranged from 0 to 43 fleas on cats treated with Vectra for Cats & Kittens and from 72 to 99 fleas on cats treated with Advantage (data not shown). The geometric mean flea counts for cats treated with Vectra for Cats & Kittens were lower than those for cats treated with Advantage at all posttreatment examinations, al-
though not all differences were statistically significant.

Only Vectra for Cats & Kittens achieved 100% efficacy at some flea counts (6 and 12 hours after treatment and on day 9). Although treatment with Advantage was never 100% effective at any post-treatment examination, its efficacy was at least 90% on days 0 (6 and 12 hours after treatment), 9, 16, 29 (12 hours after infestation) and 30.

**DISCUSSION**

Recent evidence suggests that flea-borne diseases are widespread and increasing in prevalence. Fleas can transmit a number of pathogens to cats, such as the hemotropic *Mycoplasma* spp, *Bartonella henselae*, *Rickettsia felis*, and *Dipylidium caninum*. Fleas also can cause flea allergy dermatitis and anemia in susceptible cats.

To protect cats from the constant threat of flea infestations, all Vectra products are designed to work as part of an integrated ectoparasite control tool. Safety studies show that the Vectra for Cats & Kittens formulation was very well tolerated, in both adult cats and kittens 8 weeks of age or older, at doses ranging from one to five times the recommended label dose. In this study, Vectra for Cats & Kittens demonstrated the ability to kill 98% or more of cat fleas (*C. felis*) by 6 hours after treatment and for up to 30 days.

Figure 1. Comparative speed of kill against the cat flea (*Ctenocephalides felis*) on cats weighing less than 9 lb on (A) day 0 at 2, 6, and 12 hours after treatment, (B) day 29 at 2, 6, and 12 hours after infestation, and (C) days 9, 16, 23, and 30. aSignificantly (P < .01) different from Advantage. bSignificantly (P < .05) different from Advantage.

**CONCLUSION**

The efficacy of kill of *C. felis* with Vectra for Cats & Kittens at 2 hours (83.9%) and 6 hours (100%) after treatment demonstrated in this study indicates the clinical proficiency of Vectra for Cats & Kittens for the prevention of flea infestations. The faster fleas are killed, the less
chance they have to take repeated blood meals and cause harm to cats.

**ACKNOWLEDGMENTS**

We are very grateful to Mr. Boyd Gale for the technical and organizational support during this study.

**REFERENCES**